

09/673779

1. A method for determining a bacterium suspected of being present in a sample comprising
a) testing said sample by Gram-staining and
b) testing said sample with a probe according to an in situ hybridisation protocol selected on the basis of the outcome of said Gram-staining.

2. A method according to claim 1 wherein said sample is a clinical sample.

3. A method according to claim 2 wherein said sample is mammalian blood, preferably being derived from a human.

4. A method according to claim 1, 2 or 3 wherein said Gram-staining indicates the presence of a Gram-negative bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.

5. A method according to claim 4 wherein said character is of the rod type, further comprising hybridising said sample with at least one probe selected from a group of probes capable of hybridising with nucleic acid found in *Escherichia coli*, in *Klebsiella pneumoniae*, in *Klebsiella oxytoca*, in *Serratia marcescens*, in *Enterobacter aerogenes*, in *Enterobacter cloacae*, in *Proteus vulgaris*, in *Proteus mirabilis*, in *Salmonella typhi*, in *Pseudomonas aeruginosa*.

6. A method according to claim 5 wherein said nucleic acid is ribosomal RNA.

7. A method according to claim 6 wherein said probe is having no more than five, preferably no more than two mismatches with a probe selected of a group composed of probes having a sequence GCCTGCCAGTTTCAAATG or GTAGCCCTACTCGTAAGG or GAGCAAAGGTATTAACTTACTCCC or GTTAGCCCGTCCCTTTCTGG.

8. A method according to claim 4 wherein said character is of the coccus type, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme

staining indicates the presence of Gram-positive

bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.

10. A method according to claim 9 wherein said character is of the rod type, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme and/or Proteinase K.

11. A method according to claim 9 wherein said character is of the coccus type, further comprising determining a chain-like or clump-like character of said bacteria.

12. A method according to claim 11 wherein said character is chain-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme.

13. A method according to claim 12 further comprising hybridising said sample with at least one probe selected from a group of probes capable of hybridising with nucleic acid found in *Enterococcus faecalis*, in *Streptococcus pneumoniae*, in *Streptococcus mitis*, in *Streptococcus viridans*, in *Streptococcus sanguis*, in *Enterococcus faecium*.

14. A method according to claim 13 wherein said nucleic acid is ribosomal RNA.

15. A method according to claim 14 wherein said probe is having no more than five, preferably no more than two mismatches with a probe selected of a group composed of probes having a sequence TTATCCGCTGTGATGGG or AGAGAAGCAAGCTTCTCGTCCG or GCCACTCCTCTTTCCGG.

16. A method according to claim 11 wherein said character is clump-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysostaphin and/or Proteinase K.

17. A method according to claim 16 further comprising hybridising said sample with at least one probe selected from a group of probes capable of hybridising with nucleic acid found in *Staphylococcus aureus*, in *Staphylococcus haemolyticus*, in *Staphylococcus saprophyticus*.

RECHTSREGIOEN
VEREENIGDE DEN Haagse RECHTSREGIOEN
NL 59489 21.10
NL U09900223

19. A method according to claim 18 wherein said probe is having no more than five, preferably no more than two mismatches with a probe selected of a group composed of probes having a sequence GCTAATGCAGCGCGGATCC or CCGAAGGGAGGGCTCA or AGAGAAAGCAAGCTTCTCGTCCGTT.
20. A method according to any of claims 4 to 19 further comprising hybridising said sample with at least one positive control probe and/or with at least one negative control probe.
21. A method according to claim 20 wherein said positive control probe comprises no more than five mismatches with a probe with the sequence GCTGCCTCCCCGTAGGAGT and/or wherein said negative control probe comprises no more than five mismatches with a probe with the sequence ACTCCTACGGGAGGCAGC.
22. A method according to anyone of claims 1 to 21 further comprising a one-step procedure to bind bacteria present in said sample to a microscopic slide and simultaneously fix intracellular structures.
23. A method according to anyone of claims 1 to 22 wherein said probe is selected for its reactivity with one or a group of bacterial genera and/or species having congruent susceptibility to antibiotic treatment.
24. A probe detecting or identifying a bacterium in a sample, preferably a clinical sample, said probe designed to hybridise specifically with nucleic acid in bacteria with congruent susceptibility or resistance to antibiotics.
25. A probe according to claim 24 wherein said probe is having no more than five, preferably no more than two mismatches with a probe selected of a group composed of probes having a sequence GCCTGCCAGTTTCGAATG or GTAGCCCTACTCGTAAGG or GAGCAAAGGTATTAACCTTACTCCC or GTTAGCCGTCCCTTCTGG or TTATCCCCCTCTGATGGG or AGAGAAAGCAAGCTTCTCGTCCC or GCCACTCCTCTTTCCGG or GCTAATGCAGCGCGGATCC or CCGAAGGGAGGGCTCA ~

26. A diagnostic test kit comprising means for detecting or identifying a bacterium suspected of being present in a sample using a method according to anyone of claims 1 to 23 or using a probe according to claim 24 or 25.